

REMARKS

Applicants have received and reviewed the Office Action dated November 20, 2002. By way of response, Applicants have amended claims 1, 17 and 26. Claims 1-21, 26-32, 35 and 36 are pending. No new matter is introduced.

For the reasons given below, Applicants respectfully submit the newly presented claims are in condition for allowance, and notification to that effect is earnestly solicited.

Rejection of Claims Under § 112, second paragraph and § 101

The Examiner rejected claims 1-21 under 35 U.S.C. § 112, second paragraph and §101. The rejected claims have been amended.

Amended independent claims 1, 17 and 26 now recite active steps in the claimed method.

Accordingly, it is believed that the newly presented claims fully comply with § 112, second paragraph. Withdrawal of the rejection is respectfully requested.

Rejections Under § 103(a)

The Examiner rejected claims 1-21, 26-32, 35 and 36 as being unpatentable over Rohrig et al. ("Growth of Tobacco Protoplasts Stimulated by Synthetic Lipo-chitooligosaccharides," Science, Vol. 269, Aug. 1995, pp. 841-843). Applicants respectfully traverse.

First, the Rohrig et al. reference relates to research performed at the cellular, not whole plant, level. The results of *in vitro* stimulation of mutated or non-mutated tobacco protoplasts with synthetic LCOs does not teach or suggest to one of skill in the art that LCOs can enhance seed germination, seedling emergence, plant growth or the breaking of dormancy in legume or non-legume plants. While Rohrig et al. indicate that LCO can act as a phyto-hormone by activating the cell division of tobacco protoplasts in the absence of either auxin or cytokinin, the artificial growth conditions described in Rohrig cannot be said to simulate normal plant growth conditions. Thus, there is no indication in Rohrig et al. that one of skill in the art would be successful in increasing coordinated and normal growth of whole plants or breaking the dormancy of whole plants grown under normal field conditions.

Applicants respectfully disagree with the Examiner's contention that one having ordinary skill in the art would be motivated to determine the optimum amount of LCO to use to promote plant growth. The findings of Rohrig et al. are confined to a single species in an artificial growth

system. Clearly, concentrations as determined by an artificial cell culture system cannot be viewed as predictive of concentrations to be used on whole plants when grown in the laboratory or under field conditions. The instant application demonstrates the effects of the compositions in both laboratory and under field conditions. This is important because clear laboratory findings only obviate the need for field research to determine whether the findings will stand up in the field. Often, they do not. Thus, clearly, a cell-based assay cannot and should not be interpreted as teaching the methods of the present invention.

Second, the Rohrig et al. reference was discredited in the scientific community prior to the filing date of the present application. In March of 1999, evidence emerged that the data reported in the article was falsified and it was demonstrated that the results are not reproducible. See attached Exhibits A, B and C. Clearly, because this reference has been proven fraudulent, one of skill in the art would not rely upon it. Further, one of skill in the art would have absolutely no reasonable expectation of success in adapting the findings for even *in vitro*, much less whole plant, applications. Therefore, Applicants assert that Rohrig et al. is not properly applied against the present application.

For these reasons, Applicants respectfully submit that the claims are in condition for allowance and notification to that effect is earnestly solicited.

Summary

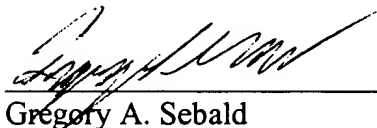
In summary, each of claims 1-21, 26-32, 35 and 36 are in condition for allowance. The Examiner is invited to contact Applicants' undersigned representative at the telephone number listed below, if the Examiner believes that doing so will expedite prosecution of this patent application.

Respectfully submitted,

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4/19/02



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VERSION WITH MARKINGS TO SHOW CHANGES MADE

Claims 1, 17 and 26 were amended as follows.

1. (AMENDED) A [M]method for enhancing plant crop seed germination, [and/or] seedling emergence [and/]or growth of a plant crop comprising the steps of:

[a treatment] providing [in the vicinity of one of a seed, root or plant with] a composition [which] that comprises an effective amount of at least one lipo chitooligosaccharide (LCO) [together with] and an agriculturally suitable carrier[,]; and

applying the composition in the vicinity of a seed, root or plant [wherein said] in an effective amount for [enhances] enhancing seed germination, [and/or] seedling emergence [and/or] or growth of said plant in comparison to an untreated plant.

17. (AMENDED) A method for breaking the dormancy [and/]or quiescence of a plant comprising the steps of:

[a treatment] providing [in the vicinity of a seed, tuber, or root of said plant with an effective amount of] an agricultural composition comprising at least one lipo chitooligosaccharide (LCO) [together with] and an agriculturally suitable carrier[,]; and

applying the composition in the vicinity of a seed, tuber or root [wherein said] in an effective amount to enable[s] a breaking of the dormancy [and/]or quiescence of said seed, tuber, or root, in comparison to an untreated seed, tuber, or root.

26. (AMENDED) A method for enhancing seed germination, [and/or] seedling emergence [and/]or growth of a plant crop comprising the steps of:

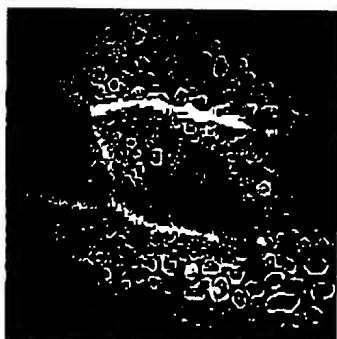
[an incubation of] providing a rhizobial strain which expresses a lipo chitooligosaccharide (LCO); and

incubating the rhizobial strain in the vicinity of one of a seed [and/]or root of said plant such that said LCO enhances seed germination, [and/or] seedling emergence [and/]or growth of said plant crop, wherein said [inoculation] incubation enhances seed germination, [and/or] seedling emergence [and/]or growth in comparison to a non-inoculated seed [and/]or root of said plant.

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Front cover: Accumulation of vesicles containing autofluorescent feruloyl-methoxytyramine and other hydroxycinnamic acids in onion bulb scale epidermal cells. Fleishy bulb scales were treated with an elicitor derived from cultures of *Botrytis allii* grown on onion cell walls. In this issue, McLusky *et al.* (pp. 523-534) describe the accumulation of granular deposits of feruloyltyramine derivatives as part of a defence response at sites of attempted penetration by the fungus. The response is associated with highly localized increases in peroxidase activity in the cell wall, polarisation of actin microfilaments and widespread suppression of flavonoid biosynthesis. The onion epidermal cell system is now an attractive model for analysis of intra- and intercellular control of vesicle synthesis, trafficking and exocytosis in plants.

EXHIBIT

A

The Plant Journal (1999) 17(5), 461–466

The following paper was submitted to *The Plant Journal*. After peer review and revision it was accepted. Following the discovery of scientific fraud in the Department of Genetic Principles of Plant Breeding at the Max Planck Institute for Plant Breeding in Köln, a wide-ranging group of researchers was assembled to repeat some key experiments. The outcome, described in the paper below, is that the published data from the Köln MPI on phytohormone-independent cell division were not reproducible. This concerns papers dating back to 1992.

Re-evaluation of phytohormone-independent division of tobacco protoplast-derived cells

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Summary

We have used a [³H] thymidine incorporation assay and microscopic observation in order to reassess recently published data dealing with the response of tobacco protoplasts to phytohormones, lipochitooligosaccharides and peptides (Harling *et al.*, 1997; Hayashi *et al.*, 1992; Miklashevichs *et al.*, 1996; Miklashevichs *et al.*, 1997; Röhrig *et al.*, 1995; Röhrig *et al.*, 1996; van de Sande *et al.*, 1996; Walden *et al.*, 1994). These proliferation assays reveal that, in contrast to published data, isolated cells of the investigated mutant plant lines axi159 (Hayashi *et al.*, 1992; Walden *et al.*, 1994), axi4/1 (Harling *et al.*, 1997) and cyi1 (Miklashevichs *et al.*, 1997), which were generated by activation T-DNA tagging, were unable to grow in the absence of auxin or cytokinin. Furthermore, lipochitooligosaccharides which play a key role in the induction of nodules on roots of legumes were unable to promote auxin- or cytokinin-independent cell division in tobacco

protoplasts as claimed by Röhrig *et al.* (1995, 1996). The finding of van de Sande *et al.* (1996) that *ENOD40* confers tolerance of high auxin concentration to wild-type tobacco protoplasts was also reinvestigated. The results of our investigations show that we were unable to reproduce the proliferation data presented in this study, which were obtained by counting tobacco protoplast-derived cells undergoing division. In total, none of the published data on phytohormone-independent division of tobacco cells could be reproduced.

Introduction

This paper reports on the use of a new assay to re-examine earlier published work dealing with the hormonal control of tobacco cell division (Harling *et al.*, 1997; Hayashi *et al.*, 1992; Miklashevichs *et al.*, 1996; Miklashevichs *et al.*, 1997; Röhrig *et al.*, 1995; Röhrig *et al.*, 1996; van de Sande *et al.*, 1996; Walden *et al.*, 1994). In these papers, the division frequency of isolated tobacco cells was preferentially determined by microscopic counting of cells undergoing proliferation. In order to independently test these data, we used cell division assays which are based either on the incorporation of [³H] thymidine into the DNA of proliferating protoplast-derived cells or on the ability of dividing cells to form microcalli after embedding in agarose. Here we report on the reproducibility of data in the published work in question.

Results and discussion

Cultured tobacco protoplasts have been used to dissect the response of plant cells to phytohormones, peptides and lipochitooligosaccharides (LCOs) as a novel class of plant growth regulators. Under defined culture conditions, these cells proliferate and form calli when the phytohormones auxin and cytokinin are added to the medium. The aim of these publications brought into question was to identify growth factors or genes that promote phytohormone-independent cell division. To assess the effect of the expression of such genes on the phytohormone response, the proliferation of wild-type protoplast-derived cells in

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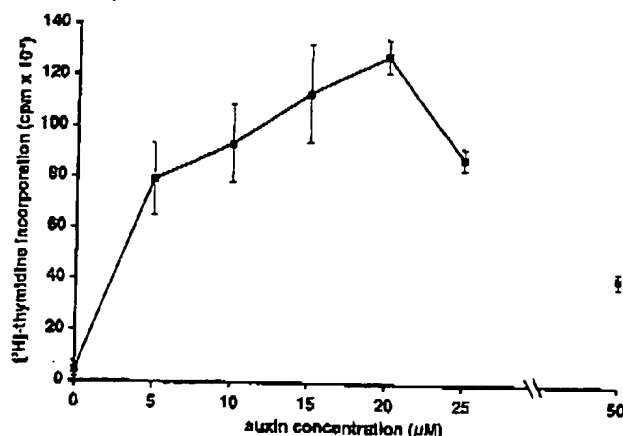


Figure 1. Effect of different auxin concentrations on the proliferation of tobacco mesophyll cells.

Protoplast-derived cells (5×10^5) were incubated in the presence of $1 \mu\text{M}$ kinetin and varying amounts of 1-NAA for 5 days. After pulse labelling with [^3H]-thymidine for 24 h, the [^3H]-thymidine incorporation into DNA of isolated tobacco cells was assayed as described in Experimental procedures. This experiment was repeated twice with similar results.

the absence of externally supplied phytohormones was compared to that of transfected protoplasts or protoplasts made from transgenic plants. Since there is a close correlation between cell doubling and DNA synthesis, we used the incorporation of tritiated thymidine into cellular DNA as an indirect parameter of cell proliferation. This [^3H] thymidine incorporation assay was developed and used in addition to the assay which had been used throughout the previously published work, and which was based on the counting of dividing cells.

Effect of different auxin concentrations on cell division

Protoplasts were incubated in the presence of $1 \mu\text{M}$ kinetin and different concentrations of auxin ranging from 5 to $50 \mu\text{M}$ of 1-NAA. After 5 days, the cells were pulse-labelled with [^3H] thymidine for 24 h, and the incorporation of radioactivity into trichloroacetic acid (TCA) precipitable material was determined by scintillation counting. As expected, the addition of auxin and cytokinin to cultured wild-type protoplasts synergistically activated DNA synthesis in these cells (Figure 1). Cytokinin alone was not sufficient to stimulate the incorporation of radioactivity into cellular DNA. The results obtained by the [^3H] thymidine incorporation assay correlated well with data obtained by microscopic visualisation of tobacco cells and were essentially similar to those obtained by evaluating the number of calli which appeared after the embedding of dividing protoplast-derived cells in agarose (data not shown). The auxin dose-response curve (Figure 1) showed that division in wild-type tobacco cells is stimulated by a relatively broad range of auxin concentrations ($5\text{--}25 \mu\text{M}$ of 1-NAA). A significant sensitivity to higher levels of auxin in the medium was only observed in the presence of $50 \mu\text{M}$

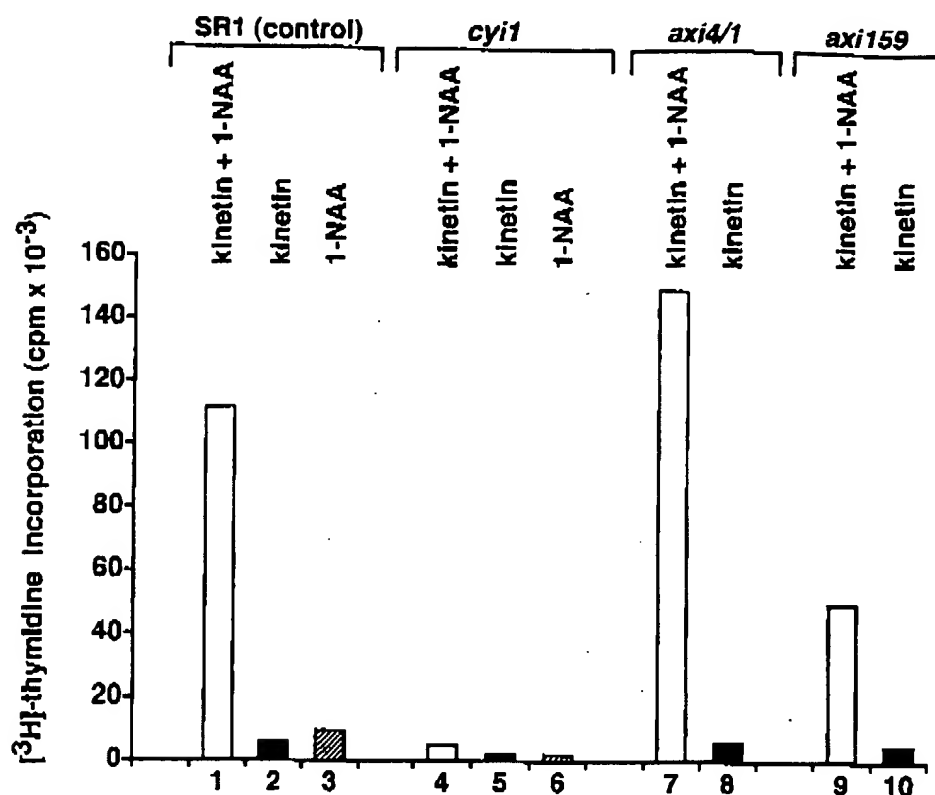
1-NAA. These findings are in contrast to data presented in previous publications (Ichikawa *et al.*, 1997; Miklashevichs *et al.*, 1997; van de Sande *et al.*, 1996; Walden *et al.*, 1994), which reported that wild-type (SR1) tobacco cells require a defined amount of $5 \mu\text{M}$ 1-NAA for optimal cell division. Furthermore, the considerably diminished division frequency of tobacco cells in the presence of $13.8 \mu\text{M}$ of 1-NAA shown in these papers is not in line with the present findings.

Proliferation of 'mutant tobacco cells' generated by activation T-DNA tagging

In several publications, activation T-DNA tagging was used to generate three different mutant cell lines from which plants were regenerated by selecting for growth in the presence of hygromycin and in the absence of externally supplied phytohormones (Harling *et al.*, 1997; Hayashi *et al.*, 1992; Miklashevichs *et al.*, 1997; Walden *et al.*, 1994). This method is based on the use of a T-DNA tag which contains multiple transcriptional enhancers (Walden *et al.*, 1991). The integration of the tag into the plant genome should produce a dominant mutation which leads to a deregulated expression of flanking plant genes. Using activation T-DNA tagging, cytokinin-independent mutants of *Arabidopsis* have indeed been generated which allow the identification of a gene involved in cytokinin signal transduction (Kakimoto, 1996), thus confirming the general validity of the activation gene tagging approach. However, it was reported that cultured cells isolated from the tagged mutant plants *cyi1* (Miklashevichs *et al.*, 1997), *axi4/1* (Harling *et al.*, 1997) and *axi159* (Hayashi *et al.*, 1992), in which the gene *AX1* was tagged (Walden *et al.*, 1994), were able to divide in the absence of externally supplied auxin. In addition, protoplast-derived cells from *cyi1* plants were claimed to divide without exogenously added cytokinin and auxin (Miklashevichs *et al.*, 1997). To re-evaluate these results, wild-type tobacco SR1 and mutant plant lines *cyi1*, *axi4/1* and *axi159* were grown as described under Experimental procedures. From 8-week-old plants, protoplasts were isolated and analysed for their phytohormone requirement. The data from the [^3H] thymidine incorporation assays clearly show that tobacco cells from *cyi1* mutant plants were unable to proliferate in the absence of auxin or cytokinin. Interestingly, these cells did not divide even in the presence of both phytohormones during the assay period described (Miklashevichs *et al.*, 1997). Furthermore, cells isolated from transgenic *axi4/1* and *axi159* tobacco plants did not incorporate [^3H] thymidine in the absence of auxin (Figure 2). In addition, growth and division was scored by microscopic evaluation of the cells 3, 5, 7 and 9 days after preparation of protoplasts and by the ability of dividing plant cells to form microcalli. The results from these analyses confirmed that the investigated cell lines

Figure 2. Proliferation of mutant cells generated from the tobacco cell lines *cyi1*, *axi4/1* and *axi159*.

Cells were grown in the presence of growth factors as indicated at the top of the figure. Concentrations of phytohormones in the media were: 1 μ M kinetin plus 5 μ M 1-NAA (open bars), 1 μ M kinetin alone (solid bars), 5 μ M 1-NAA alone (hatched bars). Conditions and measurements of [3 H]-thymidine incorporation were as in Figure 1. This experiment was repeated twice with similar results. Standard deviations were, on average, 13% of the means shown here.



were unable to divide in the absence of externally supplied auxin or cytokinin (data not shown).

Functional analyses of DNA which was tagged in 'mutant plants'

In publications by Hayashi *et al.* (1992), Harling *et al.* (1997) and Miklashevichs *et al.* (1997) it was reported that overexpression of plant DNA sequences flanking the T-DNA tag in the plant genome is responsible for the so-called 'phytohormone-independent' growth of the mutant tobacco cells. To demonstrate this, plant DNA carrying the T-DNA tag, together with an origin of replication and an antibiotic marker gene functional in *Escherichia coli*, was rescued as described by Walden *et al.* (1991). It was claimed that this rescued plant DNA was able to confer phytohormone-independent cell division to wild-type tobacco protoplast-derived cells after polyethylene glycol (PEG)-mediated transfection. To re-analyse the biological activity of the rescued sequences, PEG-mediated DNA uptake experiments were performed following the protocols published in the original papers. The rescued plasmids, pHH159 derived from 'mutant' plant *axi159*, p19En4 from *axi4/1*, and pCY11 from *cyi1* were transfected into SR1 protoplasts. These transfected cells were then tested for their ability to divide in the absence of auxin or cytokinin. Our results show that, in contrast to earlier claims, cells transfected with rescued DNA were unable to divide and form microcalli in the absence of externally supplied phyto-

hormones. Furthermore, Hayashi *et al.* (1992), Harling *et al.* (1997) and Miklashevichs *et al.* (1997) used the rescued plant DNA as hybridization probes to isolate full length cDNAs corresponding to the regions in question which were subsequently subcloned into expression vectors. We re-introduced these constructs into wild-type protoplasts by transfection and found that the cDNA constructs could not confer phytohormone-independent growth to tobacco cells (data not shown).

Effect of LCOs on growth of tobacco cells in the absence of auxin or cytokinin

In the paper by Röhrig *et al.* (1995), it was reported that synthetic lipochitooligosaccharides (in a concentration range of 10^{-7} to 10^{-16} M) alleviate the requirement for auxin and cytokinin to sustain the growth of tobacco cells. The LCOs synthesized *in vitro* in these experiments were biologically active, as confirmed by their ability to deform root hairs of vetch which is a specific bioassay for nodulation factors (Heidstra *et al.*, 1994).

To test the response of non-leguminous plant cells to LCOs, we isolated protoplasts from wild-type tobacco and cultured these cells in the presence of LCOs but in the absence of kinetin or 1-NAA. The results presented in Figure 3 show that LCOs added at a concentration of 10^{-7} M to cultured tobacco cells could replace neither auxin nor cytokinin. Furthermore, Northern blot data presented in the paper by Röhrig *et al.* (1995), showing that LCOs and

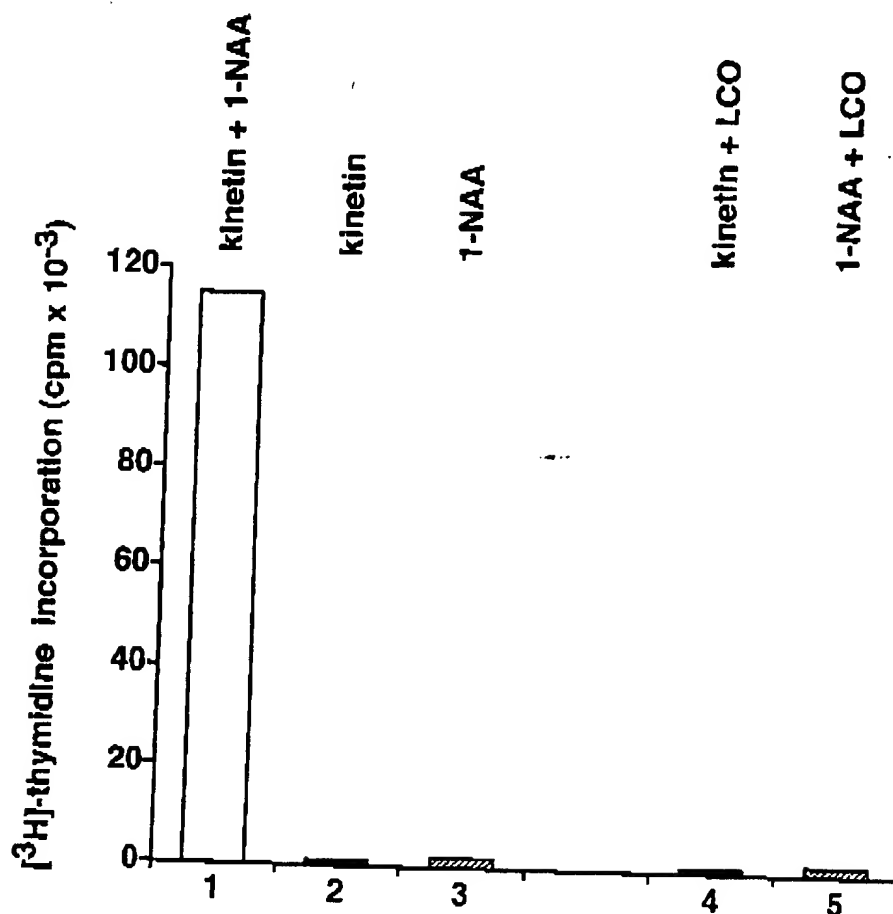


Figure 3. Effect of synthetic LCOs on the proliferation of isolated SR1 cells. The response of cultured tobacco cells to 10^{-7} M of *N-trans*-9-octadecenyl GlcN (β -1-4-GlcNAc)₃ in the presence of 1 μ M kinetin (solid bars) or 5 μ M 1-NAA (hatched bars) was determined using the [³H]-thymidine incorporation assay. As control, isolated tobacco cells were grown in a medium containing both phytohormones (open bar). This experiment was repeated twice with similar results.

auxin activate the expression of the *AX11* gene in isolated tobacco cells, could not be reproduced (data not shown).

To test the putative effects of different growth factors on *AX11* expression, we also used the chimeric *P_{AX1}-GUS* expression plasmid in which the reporter gene *GUS* was fused to the *AX11a* promoter from *Arabidopsis thaliana* (Röhrig *et al.*, 1996). Tobacco protoplasts transfected with the reporter gene plasmid were incubated in the presence of kinetin at 10^{-6} M and additionally with LCOs at 10^{-7} M or 1-NAA at 5×10^{-6} M. After 48 h, neither LCOs nor 1-NAA was found to stimulate transient *GUS* expression (data not shown), which is in agreement with the above data showing that neither LCOs nor auxin are able to induce the *AX11* promoter.

Do plant cells release a peptide as a mediator of the auxin signal?

Recent work by Miklashevichs *et al.* (1996) has suggested that auxin-induced division of plant cells might be mediated by a peptide. In this paper, the putative anti-auxin, β -naphthalene acetic acid (2-NAA; Macdonald *et al.*, 1991; Watahiki *et al.*, 1995) was used to inhibit auxin-triggered cell division. Using the [³H] thymidine proliferation assay, we analysed the effect of different 2-NAA concentrations

on the division of isolated tobacco cells in the presence of kinetin. The results showed that 2-NAA stimulates cell growth as well as the auxin 1-NAA and thus cannot be used to inhibit auxin-induced proliferation of tobacco cells (data not shown). The experiments published by Miklashevichs *et al.* (1996) showed that, in response to the 1-NAA treatment, tobacco cells might release a second growth-promoting factor (presumably a peptide) that mediates the growth-promoting effects of auxin. Since 2-NAA was the basic tool to inhibit cell division in these investigations, the data presented by Miklashevichs *et al.* (1996) are questionable.

Does the ENOD40 peptide modify the auxin response of tobacco cells?

It has been demonstrated that *ENOD40* encodes a small peptide and that overexpression of *ENOD40* in tobacco plants leads to the formation of additional side shoots (van de Sande *et al.*, 1996). Furthermore, it was reported that *ENOD40* peptides from legumes and tobacco confer tolerance of high auxin concentrations to tobacco protoplast-derived cells. In this study, the inhibitory level of auxin in the assay was defined to be 13.8 μ M of 1-NAA. However, tobacco cells exhibit optimal rates of cell proliferation

between 5 and 25 μM of auxin as measured by [^3H] thymidine incorporation (Figure 1) and, therefore, the response of tobacco cells to *ENOD40* was re-investigated. The auxin response of protoplasts generated from wild-type tobacco and transgenic plants expressing soybean *ENOD40* was compared. Second, the effect of the addition of synthetic tobacco and soybean peptides, as well as the transient expression of *ENOD40* on the proliferation of tobacco cells was measured. Using the [^3H] thymidine-based cell division assay, we were unable to reproduce the proliferation data which had been obtained by counting tobacco cells undergoing division (data not shown).

Conclusion

Our data on [^3H] thymidine incorporation show that auxin and cytokinin are required for the cell division of so-called axi and cyi mutants and, although one can tag genes by 'gain of function' T-DNA tagging, this did not lead to the isolation of phytohormone-independent mutants. Although small peptides encoded by the *ENOD40* gene may play a very important role in plant development, our data do not support the notion that these small peptides render tobacco cells insensitive to high concentrations of auxin.

Experimental procedures

Plant material and DNA

Nicotiana tabacum Petit Havanna SR1 (Maliga *et al.*, 1975) was used as the wild-type plant. Mutant plants representing a homozygous F3 generation of axi159 (Hayashi *et al.*, 1992), axi4/1 (Harling *et al.*, 1997) and cyi1 (Miklashevichs *et al.*, 1997) were grown in the presence of hygromycin (15 mg l⁻¹) and correspond to those used in the published work. The homozygous F4 generation of mutant 11S plants expressing 35S-*GmENOD40-2* (van de Sande *et al.*, 1996) were selected on methotrexate (0.1 mg l⁻¹).

Plant DNA was prepared as described previously (Edwards *et al.*, 1991) and analysed for the presence of T-DNA by PCR using the following primer combinations:

- (i) 5' - GAT ATC TAG ATC CGA AAC TAT CAG - 3' and
5' - GTG ATA GAT CAT ACG TAG GTC GAT - 3' for axi159
- (ii) 5' - CTT CAA TCG TTG CGG TTC TGT CAG - 3' and
5' - GCA TTC AGT GCT GCA CAG CAG AG - 3' for axi4/1
- (iii) 5' - GAT ATC TAG ATC CGA AAC TAT CAG - 3' and
5' - GAT ATC TAG ATC CGA AAC TAT CAG - 3' for cyi1.

Specific amplification of sequences containing T-DNA and flanking plant sequences resulted in unique fragments of 0.9 kb for the mutant axi159, 2.2 kb for axi4/1, and 1.2 kb for cyi1.

DNA of rescued plasmids derived from axi and cyi mutants was analysed by restriction digestion and compared with data presented in the original publications.

Cell culture

Mesophyll-protoplasts isolated from *Nicotiana tabacum* SR1 leaf tissue (Maliga *et al.*, 1973) were washed twice in K3 medium (Nagy

and Maliga, 1976) containing 0.4 M sucrose and resuspended in the same medium to a final density of 10⁵ cells ml⁻¹. Growth-promoting compounds like α -naphthalene acetic acid (1-NAA, Sigma), β -naphthalene acetic acid (2-NAA, Sigma), cytokinin (kinetin, Sigma) and synthetic LCO (*N-trans*-9-octadecenoyl GlcN [β -1,4 GlcNAc]₃) were added as indicated. Tobacco protoplasts were cultured in the presence of growth factors for 2 days in the dark at 26°C, followed by 3 days in continuous dim light (26°C).

Cell proliferation assays

Fifty μl of a diluted [^3H] thymidine solution (5 μCi) was added to isolated tobacco cells cultured for 5 days, and cells were re-incubated for 24 h. The diluted [^3H] thymidine solution (100 μCi ml⁻¹) was prepared from a 1 mCi ml⁻¹ stock (Biotrend) by dilution 1:10 with a sterile unlabelled thymidine solution (10⁻⁵ M). After radiolabelling of the cells, the suspension was diluted 1:1 with the washing solution W5 (Menczel *et al.*, 1981) and cells were harvested by centrifugation (Hettich centrifuge, 1000 g for 7 min). The supernatant was removed and cells were resuspended in 1 ml of ice-cold 5% TCA. The tubes were kept on ice for 30 min. To remove acid-soluble radioactivity, cells were centrifuged and washed separately with cold 5% TCA and absolute ethanol. Wet cell pellets were then solubilized in 500 μl of 0.1 M NaOH containing 0.2% SDS for 30 min at 37°C. Radioactivity incorporated into TCA-insoluble material was determined by scintillation counting using an acidified scintillation cocktail.

In addition to the assay based on [^3H] thymidine incorporation into the DNA of proliferating cells, division of isolated tobacco cells was evaluated microscopically 5 days after the addition of growth factors to the medium.

Alternatively, the ability of dividing cells to form microcalli within agarose sectors floating in liquid medium (Negrutiu *et al.*, 1987) was used as a further method to measure cell proliferation.

Transient expression assays

Protoplasts were transfected with 10 μg of different DNA-constructs using PEG4000 treatment (Negrutiu *et al.*, 1987). To monitor the activation of the *AX11* promoter, cells were transfected with the *P_{ax}-GUS* plasmid and after 48 h transiently expressed GUS was determined as described by Jefferson *et al.* (1987).

Acknowledgements

We thank Elizabeth Schell for comments and critical reading of the manuscript.

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SW MAIN PAGE

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SCIENTIFIC FRAUD: AN IMPORTANT CASE IN PLANT BIOLOGY

Here is an interesting case of scientific fraud, albeit with the appearance of an opera surrounding a farce:

1) A laboratory invents a new important technology which is validated by other laboratories and becomes widely used.

2) The inventing laboratory then proceeds to apply the technology to a particular problem. In the course of the application, one of the laboratory researchers apparently fabricates data to achieve important results, and a number of "co-authors" in the laboratory join with the fabricating researcher to publish the results in a series of papers in various leading international journals.

3) Ultimately, the results cannot be replicated by others, the results are considered fabrications, and the group leader of the laboratory and the fabricating researcher are forced to resign. The co-author fabricating researcher is identified as a "technician" (see the related background report below).

4) A research team led by the head of the department that housed the laboratory takes on the task of determining which published papers are suspect, and reports that 10 papers in various leading journals (Science, Nature, EMBO Journal, Proc. Natl. Acad. Sci. US, etc.), published between 1992 and 1998, probably contain serious fabrications.

5) The former group leader and the involved laboratory researcher-technician both refuse to comment on the case.

6) Two of the 10 papers have already been retracted by other co-authors, but there are apparently no plans by any co-authors to retract the other papers.

7) The editors of the various leading journals say they have no plans to retract the remaining published papers themselves, but will wait for retractions by the authors. The journal editors have evidently been criticized because at least some of the fabrications might have been detected by proper review of the papers when they were originally submitted.

The laboratory in question is part of the Max Planck Institute for Plant Breeding Research in Cologne, DE. The group leader of the laboratory was Richard Walden, who resigned in 1998, and who is apparently now working in a laboratory in the UK. The implicated "technician" is Inge Czaja. The research involved a new technique to study the actions of plant genes (activation T-DNA tagging), a validated technique now widely used in plant biology. The investigating team consisted of researchers at the Cologne institute and colleagues from other European laboratories, the team led by Jeff Schell, head of the department containing the fabricating laboratory. The investigation report concerning the 10 suspected fraudulent papers was recently published in the March 1999 issue of Plant Journal. Alan Jones (University of North Carolina, Chapel Hill US), a researcher in the field, is quoted as saying: "I can no longer believe any parts of the data in any parts of the papers... When the papers came out, I was extremely enthusiastic." Jones states that the discovery of the fabrications will have a "negative effect on the field" because major conclusions were drawn from the papers.

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Michael Balter: Data in key papers cannot be reproduced.
(Science 26 Mar 99 283:1987)

QY: Michael Balter [science_editors@aaas. rg]

Summary by SCIENCE-WEEK [http://scienceweek.c m] 4Jun99

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SW MAIN PAGE

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AGNET APRIL 5, 1999

A potential phosphate crisis
 Medfly larvae hit Florida via plane
 Plant science: data in key papers cannot be reproduced
 EU plans for large scale GM disaster
 Agbiotech World Forum 99; June 9-11, 1999 in Los Vegas, NV
 German authority's decision has organic manufacturers and exporters
 breathing sighs of relief
 This library has deep roots on the farm
 Commercial use of Burkholderia cepacia
 Environmentalists call 'beetlemania' a hollow excuse for cutting trees

Agnet is produced by researchers at the Agri-Food Risk Management and Communications Project at the University of Guelph, is edited by Douglas Powell (dpowell@uoguelph.ca), Sarah Grant (segrant@uoguelph.ca) and Amanda Whitfield (awhitfie@uoguelph.ca), and is sponsored by the Ontario Ministry of Agriculture, Food and Rural Affairs Plants Program at the University of Guelph, with additional support provided by the U.S. National Pork Producers, the U.S. National Food Processors Association, Novartis Seeds, Dairy Farmers of Canada, AGCare (Agricultural Groups Concerned About Resources and the Environment), Monsanto Canada, Pioneer Hi-Bred Limited (Canada), the U.S. National Cattlemen's Beef Association, Ontario Agri-Food Technologies, Novartis Crop Protection Canada, Dairy Farmers of Ontario, the Rutgers University Food Safety Extension Program, the Crop Protection Institute, Eastern Region Agriculture and Agri-Food Canada, Ag-West Biotech, Capital Health, the Ontario Soybean Growers Marketing Board, the Canadian Cattlemen's Association, Food Industry Environmental Network, Dow AgroSciences, W.G. Thompson & Sons, Crop and Food Research New Zealand, and the Agricultural Adaptation Council (CanAdapt Program).

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A POTENTIAL PHOSPHATE CRISIS

March 26, 1999

Science, Volume 283, Number 5410, p 2015

Philip H. Abelson

Phosphate is a crucial component of DNA, RNA, ATP, and other biologically active compounds. Microbes, plants, and animals, including humans, cannot exist without it. But, the story says, resources are limited, and phosphate is being dissipated. Future generations ultimately will face problems in obtaining enough to exist.

The current major use of phosphate is in fertilizers. Growing crops remove it and other nutrients from the soil. Long-term research at the Morrow agricultural plots of the University of Illinois at Urbana-Champaign has corroborated the fact that even the best land loses fertility unless nutrients are replenished. At the Morrow plots, there is a threefold or greater difference in yields of corn between fertilized areas and untreated ones. Most of the world's farms do not have or do not receive adequate amounts of phosphate. Feeding the world's increasing population will accelerate the rate of depletion of phosphate reserves.

Corn seeds, which are a major source of food for cattle, swine, and poultry, contain substantial amounts of phosphate. About 75% of it is in the form of phytate, a water-insoluble compound. When the seeds sprout, enzymes are created that release phosphate from the phytate, making it available for biological activities. When seeds are fed to ruminants, bacteria in the rumen degrade some of the phytate, providing phosphate for use by the

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animals. But nonruminants such as poultry, swine, and people do not have an efficient system for making phosphate available from phytate. They excrete most of it. Ultimately, some of the phosphate excreted contributes to water pollution and eutrophication and becomes unavailable for further use.

Recent scientific research has resulted in ways to diminish the loss of phosphate. One of the methods was described at the recent AAAS annual meeting in Anaheim, California. Adolphus van Loon of Hoffman-La Roche, Basel, Switzerland, reported on research results that facilitate the release of phosphate from feed prepared for chickens and hogs. One of his colleagues conducted many successful experiments to improve the stability of phytase enzymes, which catalyze the breakup of phytate. The DNA coding for phytase that is present in thermophilic bacteria was altered to produce more

highly thermostable enzymes. These are incorporated in feed when it is initially being cooked. Experiments have demonstrated that as much as one-third of the phytate phosphate is, the story says, made available when monogastric animals are fed the improved feed. Van Loon estimated that annual sales will total as much as \$500 million.

Another approach to the phytate problem has been attempts to reduce the amount of phytate in seeds. However, studies using this approach usually found that when the phytate content was decreased substantially, the seeds did not germinate, or if they did, they did not give rise to healthy plants.

Growing and feeding experiments are currently being conducted. In one study, cited in this story, University of Missouri scientists conducted experiments involving analysis of waste from pigs fed either unmodified or low-phytate corn. Pigs who were fed the low-phytate corn showed, on average, a 37% reduction in phosphorus excreted. In growing pigs, 64% of the phosphorus in low-phytate corn was available, as compared with 10% from genetically similar corn with normal phytate levels.

The rate at which U.S. farmers will adopt low-phytate varieties of corn will depend on whether seeds also provide a combination of traits that include higher yield, increased energy for feed, and resistance to pests and herbicides. Ultimately this objective will be achieved.

MEDFLY LARVAE HIT FLORIDA VIA PLANE

April 5/99

Reuters

WASHINGTON - The U.S. Agriculture Department was cited as saying today that Mediterranean fruit fly larvae were found aboard a plane in Miami after a passenger brought coffee plants, which are prohibited from entering the United States, onto the flight after a trip to Costa Rica. Michael Shannon, an official with the USDA in Florida, was quoted as saying, "This interception may have prevented another major, costly Medfly outbreak. International travelers who bring agricultural products into the United States are playing environmental roulette with the nation's natural resources."

Since 1997, approximately \$32 million has been spent to eradicate the Medfly in Florida in Manatee, Marion, Orange, Polk and Sarasota counties. Officials are continuing to release sterile Medflies in Florida and have hired 93 additional people to beef up the fruit fly detection system in the state.

PLANT SCIENCE: DATA IN KEY PAPERS CANNOT BE REPRODUCED

March 26, 1999

Science, Volume 283, Number 5410, pp. 1987 - 1989

Michael Balter

New findings, published last week, appear, according to this story, to

confirm suspicions that several key papers in a hot area of plant development were fatally compromised by scientific fraud. The results, published in the March issue of Plant Journal, stem from an investigation at the Max Planck Institute for Plant Breeding Research in Cologne, Germany, which concluded last year that a laboratory technician falsified experiments forming the basis of 10 publications going back to 1992. The technician, Inge Czaja, and the leader of the group in which she worked, Richard Walden, resigned in early 1998 in the wake of the scandal, although Walden has never been accused of participating in the fraud.

In the Plant Journal article, a team of researchers at the Cologne institute, along with colleagues from other European labs, report on their attempts to repeat key experiments in eight papers published in Science, EMBO Journal, the Proceedings of the National Academy of Sciences (PNAS), Trends in Plant Science, and Plant Journal. The authors could not reproduce the most central findings. Two other papers from the institute, which had originally appeared in Nature and PNAS in 1997, were retracted last year by most of their authors after their findings also could not be reproduced.

----- COLOGNE INSTITUTE'S DISPUTED PAPERS

Irreproducible in the Plant Journal study:

Hayashi et al., Activation of a plant gene by T-DNA tagging:

Auxin-independent growth in vitro, Science 258, 1350 (1992).

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Walden et al., Auxin inducibility and developmental expression of ax11: A gene directing auxin-independent growth in tobacco protoplasts, EMBO Journal 13, 4729 (1994).

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Rohrig et al., Growth of tobacco protoplasts stimulated by synthetic lipo-chitooligosaccharides, Science 269, 841 (1995).

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Miklashevichs et al., Do peptides control plant growth and development?, Trends in Plant Science 1, 411 (1996).

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Van de Sande et al., Modification of phytohormone response by a peptide encoded by ENOD40 of legumes and a nonlegume, Science 273, 370 (1996).

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Rohrig et al., Convergent pathways for lipochitooligosaccharide and auxin signaling in tobacco cells, Proceedings of the National Academy of Sciences 93, 13389 (1996).

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Harling et al., A plant cation-chloride co-transporter promoting cytokinin- and auxin-independent protoplast division, EMBO Journal 16, 5855 (1997).

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Miklashevichs et al., T-DNA tagging reveals a novel cDNA triggering cytokinin- and auxin-independent protoplast division, Plant Journal 12, 489 (1997).

Retracted last year:

Ichikawa et al., Identification and role of adenylylcyclase in auxin signaling in higher plants, Nature 390, 698 (1997). [retracted: Ichikawa et al., Nature 396, 390 (1998).]

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John et al., Lipochitooligosaccharide-induced tobacco cells release a peptide as mediator of the glycolipid signal, Proceedings of the National Academy of Sciences 94, 10178 (1997). [retracted: John et al., PNAS 95 (17), 10344a (1998).]

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EU PLANS FOR LARGE SCALE GM DISASTER

April 4/99

The Independent

By Marie Woolf, Political Correspondent

EUROPEAN governments are drawing up contingency plans for a nuclear fallout-style emergency involving genetically modified organisms.

A five-point Emergency Response Plan has been formulated by the European Commission, designed to cope if genetically modified plants result in widespread illness or the death of wildlife. The draft directive, set to be adopted by ministers across Europe, includes plans to "decontaminate" affected areas and destroy plants and animals exposed to GMOs. The plan is designed to prevent a human health disaster and stop genetically modified plants breeding wildly with native species. The proposed five-point plans are similar to those used in the case of accidental nuclear leaks and will be a requirement of any new application to release genetically modified organisms once the law comes into force.

So a company wishing to plant GM seeds in Britain will have to present a detailed strategy for coping with a disaster. This must include:

1. Methods and procedures for controlling the GMOs in case of unexpected spread;
2. Methods for decontamination of the areas affected, e.g. eradication of the GMOs;
3. Methods for disposal or sanitation of plants, animals, soils, etc. that were exposed during or after the spread;
4. Methods for the isolation of the area affected by the spread;
5. Plans for protecting human health and the environment in case of the occurrence of an undesirable effect.

"The case for the need for these crops has not been thought out, but governments are already gearing up for emergency decontamination operations," said Tony Juniper, campaigns and policy director of Friends of the Earth. "This is redolent of a 1960s nuclear civil defence plan." The new directive will amend the current EU law on genetically modified crops. The proposed changes, which have Government backing, will be discussed by European environment ministers in June, but could take years to implement.

"Ministers are clearly fore-seeing major problems with GMOs, or they wouldn't be considering these action plans," said Norman Baker, the Liberal Democrat environment spokes-man. "With large farm-scale trials in the UK only days away", contingency plans were "a matter of urgency", he said.

AGBIOTECH WORLD FORUM 99; JUNE 9-11, 1999 IN LOS VEGAS, NV

April 5, 1999

from a press release

AgBiotech World Forum 99

SOUTHBOROUGH, Mass. -- IBC USA Conferences, along with its international partners, Agra Europe, Ag-West Biotech, SJH and Co, the Agbiotech Reporter and Seed & Crop Digest is excited to announce the development of IBC's AgBiotech World Forum.

This unique science-to-business program will present an important opportunity for professionals in the growing agbiotech arena to gather in one place and receive breaking information on financing and investing in agbiotech, agbiotech legal hurdles, regulatory legislation, and scientific advancements in this area.

Presentations include perspectives from the U.S.D.A., addressing government/industry partnerships available for both big and small businesses. Additionally, several companies will be speaking about the advances in the production of industrial and pharmaceutical compounds in plants. Other important talks will cover essential components for the prospects of business opportunities in Europe and Japan.

Sano M. Shimoda, President of BioScience Securities, and an AgBiotech World Forum program speaker will be addressing the changing landscape of the agbiotech community as mergers and acquisitions continue and companies

join a growing "life science" industry. "The power of agbiotech is huge - you can't afford not to understand the risks and rewards of the industry," said Shimoda.

When asked to comment on the conference and its place in the international agricultural biotechnology field, Peter McCann, President of Ag-West Biotech in Saskatchewan, Canada replied, "International trade in agricultural products such as those varieties of soy, corn and canola which have been improved through the application of modern agricultural biotechnology, is being severely restricted by lack of equivalency standards and regulatory harmonization, particularly in Europe. An understanding of the current status of regulatory issues in other parts of the world, from a non North American viewpoint, is essential for the successful development of international business." McCann will further address this issue at the June conference.

Nigel Poole of Zeneca Plant Sciences will address the latest developments in the European Union's thriving and diverse agriculture and productive biotechnology research base. "Dealing with the public relations crisis in Europe is essential for the success of transgenic technologies. Presentations at this conference will equip participants with the information they need to properly address these issues," Dr. Poole said. Additionally, participants who want to undertake joint ventures with others in the agbiotech industry will have the opportunity to make business presentations at the event's Business Development Forum on June 10. Private meeting rooms will be available for one-on-one discussions, as well. Individuals with a product, new technology or service available for joint venture development, or those who are looking for new joint venture opportunities, are welcome to apply for a speaking slot. This includes start-up agbiotech companies, new technology inventors, venture capitalists, product licensees/licensors, researchers and others in any area of agbiotech who are looking for development partners. Also invited are those looking for international trade or development ventures in agbiotech.

Additional program information can be found at www.ibcusa.com/2313. IBC USA Conferences is a wholly owned subsidiary of the publicly traded London based Informa Group, which boasts sales in more than 180 countries with correspondents and agents worldwide staffing offices in 16 countries. Informa offers "must have" business to business information through 3,500 conferences and seminars and 780 print and electronic publications. Additional information on IBC USA Conferences and its world partners is available at www.ibcusa.com and www.intbuscom.com.

GERMAN AUTHORITY'S DECISION HAS ORGANIC MANUFACTURERS AND EXPORTERS BREATHING SIGHS OF RELIEF

April 5, 1999

from a press release

SAN DIEGO -- Private Organic Certifier Quality Assurance International (QAI) Announces Accreditation by an Approved European Union (EU) Member State Authority, Leaving the Door Open for Import of QAI Certified Organic Products Into Europe

Organic manufacturers, exporters and traders across North America are breathing easier today as a result of one private certifier's good news. Quality Assurance International (QAI), a private certifier of more than 500 companies for compliance to strict organic standards, was pleasantly surprised when it received word a month earlier than anticipated that it is the first and only certification organization in North America to receive VBP accreditation for its organic verification practices. On April 1, VBP (Verein zur Begutachtung von Prüfstellen) assured QAI it was no April Fool's joke when it transmitted the VBP certificate of approval to the company's headquarters. The news means QAI certified companies and products are safely in compliance for the looming EU deadline of June 30, which mandated that importers of organic products achieve one of three

accreditation options or face a stoppage into the EU.

QAI's early approval by the German-based VBP will erase much uncertainty among organic manufacturers dependent on export to the EU market. "This is an incredible victory for expansion of organic trade," said Joseph Smillie, QAI's senior vice president. "Just weeks ago most of the organic industry was tied in knots in a race to keep a trade window pried open for organic exports to Europe. Now, an entire door has just opened wide."

QAI initiated its pursuit of option three of EU guidelines last September when it began a rigorous evaluation process with VBP. In addition to scrutinizing the company's certification documents and procedures, VBP sent an independent auditor from January 10 to 18 to QAI headquarters to review client records and into the field to inspect farms and manufacturing plants in the United States and Mexico. The auditor then filed a report with the VBP review board, which includes a German state regulatory official and organic experts. Because of the EU's complex process, QAI, its clients and many European importers, feared approval would drag on dangerously close to June 30 deadline. Thus, VBP's April 1 announcement was greeted with jubilation.

"This is great news for European importers. We have been told by Dutch regulatory officials that if a third country certifier is approved by a member state of the EU it will be accepted in the Netherlands as well," said Wim Rabbie, president of the well-known Dutch trading company Tradin. Manufacturers are hailing the decision as removal of feared trade barriers to organic products. "This vitally important step will not only allow our company to continue building business in Europe, but will pave the way for the introduction of further organic product extensions in the months ahead," said Jonathan Toase, spokesperson for Imagine Foods, Inc., which has already set up a subsidiary in the United Kingdom in anticipation of accelerated growth.

"QAI's accreditation by VBP greatly facilitates increased market opportunity while assuring the highest standards of organic integrity and consumer protection," said Gary Hirshberg, president/CEO for Stonyfield Farm, the largest US manufacturer of organic yogurt.

Smillie sees the VBP accreditation as a catalyst for greater organic commerce on several levels. "...this goes beyond the obvious advantages to QAI-certified clients. Interest among North American businesses in accessing the enormous EU market has been profound in recent years. This accreditation will enhance hundreds of companies' ability to enter the dynamic EU marketplace by eliminating the confusion and trade barriers that existed previously."

"Ultimately, the European consumer will be the prime beneficiary of this decision due to increased organic product choices," said Smillie. Quality Assurance International is one of the leading private and professional certifiers of organic foods, fibers and food products in North America. Currently, QAI independently verifies the authenticity of organic products for more than 500 certified entities and several thousand additional participating companies, such as co-packers, warehouses, packing houses, manufacturers and distributors. QAI's certification program is designed to certify every link in the organic production chain and provide assurance that the actual product destined to enter into market distribution is genuinely organic. QAI is a member of Organic Trade Association.

THIS LIBRARY HAS DEEP ROOTS ON THE FARM

April 5, 1999

USDA - ARS News Service

Next week (April 11-17) is National Library Week, and one of the world's most special libraries has "Agricultural" as its middle name. The National Agricultural Library in Beltsville, Md., is the world's largest library on the subject.

NAL is part of the Agricultural Research Service, USDA's chief scientific

arm, and the library's links to scientific research are as plain as the views from its windows. The towering, 14-story library building looks west toward the labs and research fields of the largest of ARS' 100-plus research

locations-the 7,000-acre Beltsville Agricultural Research Center.

When President Lincoln established USDA in 1862, he noted that its mission would include "to acquire and diffuse among the people of the United States

useful information on subjects connected with agriculture."

Accordingly, the first U.S. Commissioner of Agriculture, Isaac Newton, established an agricultural library in which "the most valuable works would

gradually accumulate by exchange, gift, and purchase, forming a rich mine of

knowledge."

Since that time, NAL's knowledge media have shifted from paper and ink to computers and cyberspace. Its clientele, once mainly U.S. scientists and farmers, now is global.

This "mine of knowledge" is no musty accumulation gathering dust on the bookshelves. NAL handles 220,000 requests a year from scientists, teachers,

government officials, farmers, business leaders, students and others around

the nation and the world.

The NAL treasure trove extends over 50 miles of bookshelves, with 3.3 million books, reports, databases, artifacts, audiovisuals, periodicals and

other items. Growing by upwards of 130,000 accessions each year, the library

has materials in about 75 languages.

The computer age has brought NAL new ways of doing business, including sending out most materials electronically. An extensive web site, offering access to library staff, products and services, currently receives 11 million "hits" a year. The web address is:

<http://www.nal.usda.gov>

COMMERCIAL USE OF BURKHOLDERIA CEPACIA

March-April 1999

Emerging Infectious Diseases

<http://www.cdc.gov/ncidod/eid/current.htm>

John J. LiPuma* and Eshwar Mahenthiralingam

*MCP Hahnemann University, St Christopher's Hospital for Children, Philadelphia, Pennsylvania, USA; and University of British Columbia, Vancouver, British Columbia, Canada

To the Editor: In their review of the potential threat to human health by the commercial use of *Burkholderia cepacia*, Holmes et al. (1) focus on the biopesticidal uses of this bacterium in agriculture. By virtue of its ability to antagonize a number of soilborne plant pathogens, *B. cepacia* is an attractive natural alternative to currently used chemical pesticides, such as captan, mancozeb, and metalaxyl. The replacement of these highly toxic agents, which are among the mainstays of crop protection chemicals, by safer products is a laudable goal. However, despite being nonpathogenic to healthy humans (and thus classified as a Biosafety Level 1 species), *B. cepacia* can cause life-threatening pulmonary infection in persons with cystic fibrosis. Holmes et al. call for a moratorium on the use of *B. cepacia* in agriculture until more is known about risks from such use. Perhaps of greater concern than agricultural use is *B. cepacia*'s use as a bioremedial agent. Holmes et al. only briefly refer to the capacity of this species to degrade chlorinated aromatic substrates such as those found in certain pesticides and herbicides. By virtue of its extraordinary metabolic versatility, *B. cepacia* can use such compounds as nutrient

carbon energy sources. In addition, some strains produce enzymes capable of degrading nonnutritive substrates, such as trichloroethylene (TCE), a major ground water contaminant used in the dry cleaning industry and in degreasing solvents.

The degree to which *B. cepacia* is being used in bioremediation products is unknown; however, the species has been used extensively to degrade ground water TCE contamination in at least one large U.S. city. A number of environment-friendly bioremediation products containing only naturally occurring, nonpathogenic bacteria are being marketed for use in drain opening and grease eradication systems. Because their formulations are proprietary, it is not known if these products contain *B. cepacia*; however, franchises that distribute such totally natural, noncorrosive, nontoxic products specifically target fast-food restaurants, photo processing facilities, and hospital radiology departments.

In the United States, the biopesticidal use of microorganisms such as *B. cepacia* is regulated by the Environmental Protection Agency (EPA) under the

Federal Insecticide, Fungicide, and Rodenticide Act; however, the use of naturally occurring, nonpathogenic bacteria as bioremedial agents is essentially unregulated. Only new microorganisms (i.e., intergeneric or formed by combining genetic material from organisms in different genera) are regulated by EPA under the Toxic Substances Control Act (TSCA) (2). Ironically, TSCA regulations provide a strong disincentive to the development of safer microbiologic alternatives for use in bioremediation. For example, although the genetic elements responsible for TCE degradation by *B. cepacia* have been cloned, their recombination into another nonpathogenic bacterial host (e.g., *Escherichia coli*) would constitute a new microorganism, the licensure of which would be considered prohibitively time-consuming and expensive by many companies.

In Canada, biopesticidal uses of microorganisms are regulated by the Pest Management Regulatory Agency of Health Canada, under the Pest Control Products Act (PCPA); bioremedial uses are regulated by Environment Canada under the Canadian Environmental Protection Act (CEPA) (3). Both naturally occurring and genetically engineered microorganisms are strictly controlled under these acts. However, accurate species identification is the cornerstone of all notification of products under the Canadian regulations. This presents a further dilemma. At least five genomovars (discrete species) constitute what has recently been designated the "*B. cepacia* complex" (4). Insofar as the taxonomy of this group is poorly defined, there are no conventional taxonomic designations to distinguish pathogenic from nonpathogenic species. At present, it appears that all five *B. cepacia* genomovars are capable of causing infections in vulnerable persons (4).

Because the epidemiology of *B. cepacia* complex infection in humans is incompletely understood, the threat posed by the inclusion of this species in biopesticides and bioremedial products is difficult to quantify.

However,

we agree with Holmes et al. that such use should be approached with considerable caution. In a broader context, the commercial use of *B. cepacia*

illustrates our incomplete understanding of nonpathogenic bacteria and their

potential to cause human disease. Regulations governing the use of microorganisms in industry must constantly adapt to keep pace with the emergence of infections due to nonpathogens and limit risk to human health.

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ENVIRONMENTALISTS CALL 'BEETLEMANIA' A HOLLOW EXCUSE FOR CUTTING TREES;
FORESTRY: CITING BEETLE EPIDEMICS IN SEVERAL NORTHWESTERN AREAS, THE
FOREST SERVICE WANTS TO SELL THEIR TIMBER TO LOGGERS. OPPONENTS SAY THE
INFESTATION IS FABRICATED.

Apr. 4 /99

Los Angeles Times

AP

JOHN HUGHES

WASHINGTON -- Douglas fir bark beetles are killing thousands of trees in the Pacific Northwest, and Forest Service officials, according to this story, say they can do little to stop them.

But the officials say some good can come from what they consider an epidemic. They can sell trees on 25,000 infected acres in Washington state and Idaho and use the proceeds to rebuild roads, plant more trees, protect streams and cut the risk of forest fires.

Dan Dallas, a district ranger in the Colville National

Forest in northeast Washington was quoted as saying, "What we're trying to do is realize some of the value by salvaging the lumber. I'm not going to have another opportunity like this, I'm sure, to make this many needed improvements in a road system I can't maintain."

But, the story adds, environmentalists say there is no beetle epidemic. They contend the little black-headed bug with reddish-brown wings is being used as an excuse to boost the cut and to increase Forest Service income.

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